IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	SCHLOTHAUER, et al	)	Examiner: Prats, F.C.
			)	
Appl. No.	:	09/720,041	)	Group Art Unit 1651
			)	
Filed	:	April 2, 2001	)	
			)	
For	:	BIOACTIVE WHEY PROTEIN HYDROLYSATE	)	

DECLARATION OF JULIAN ROBERT REID

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, JULIAN ROBERT REID, depose and say as follows:

1. I am New Zealand citizen and live in Palmerston North, New Zealand.

2. I am a Senior Research Scientist at Fonterra Palmerston North, New Zealand. My Curriculum Vitae is attached as Exhibit "JRR1".

3. I am an inventor of the above-identified patent application.

4. I have read the papers entitled "Angiotensin - I – converting Enzyme Inhibitory Activities of Gastric and Pancreatic Protenase Digest of Whey Proteins", by Mullally *et al*, Int. Dairy Journal 7 (1997) 299-300; and "New Derivation of the Inhibitory Activity against Angiotensin Converting Enzyme (ACE) from Sweet Cheese Whey", Abubakar *et al*, Tohoku Journal of Agricultural Research, Volume 47, Number 1-2, September 1996, 1-8; which have been cited against patent application USSN 09/720,041

5. In order to do a comparison between the hydrolysate which would have been produced using the enzyme deactivation conditions described in Mullally and the conditions defined by claim 32 of the application a technician employed by my employer carried out an hydrolysis using the conditions of example 4 of USSN 09/720,041. However, the hydrolysis was terminated through using conditions

JR.

described on page 300 of the Mullally paper, namely, inactivation by heating to 80°C for 20 minutes. I have read and refer to the declaration of Sophia Stathopoulos being filed with this declaration, which describes that hydrolysis.

6. The conclusion reached by Ms Stathopoulos and illustrated in the photographs is that the hydrolysate is of no commercial value. Although small and medium sized peptides in the hydrolysate which express ACE inhibiting activity are soluble because they possess no secondary or territory structure, the undigested protein and the very large protein fractions which have been denatured by the deactivating conditions are also present. The hydrolysate as a whole is not able to be further processed to sell as a commercial product.

7. On page 302 of the Mullally *et al* paper, at paragraph 1, left column, the authors describe having preheated substrates to produce heat denatured substrates. Heat denatured means insoluble substrates. The authors describe making denatured substrates by heating the whey protein concentrates at 80°C for 20 minutes. These are the same temperature and time conditions used by the authors for inactivation of the enzymes in the hydrolysate (see page 300, left column, lines 42, 43; right hand column, lines 3 and 4).

8. In the same paragraph 1 on page 302 it is stated that there is no difference in ACE inhibition index values between hydrolysates obtained from heat denature substrates and non-heat denatured substrates. The only conclusion I can come to as the reason for this is that after heat deactivation of the enzymes, the so called non-heat denatured substrates contain large fractions of denatured proteins which are insoluble, as was shown in the experiment carried out by Ms Stathopoulos. Although Mullally *et al* were able to test the soluble portion for bioactivity that does not mean the denatured insoluble portion was not also present as well.

9. On page 300 of the Mullally *et al* paper, right column under the heading "Ultrafiltration of Hydrolysates" it is stated that hydrolysate ultrafiltration was conducted with a stirred cell UF system. This device features a stirring bar that continuously sweeps the surface of the molecular weight cut off membrane during filtration to prevent insoluble material in the sample from settling on the membrane and thus fowling it. This is further evidence that the hydrolysates prepared under the

JR.

conditions described in the paper contained denatured and therefore insoluble material. If the Mullally hydrolysate was totally soluble the stirring bar would not have been needed.

10. In the accompanying declaration of Sophia Stathopoulos she describes how she repeated the experiment of example 4 of USSN 09/720,041. However, the hydrolysis was continued for a period of 24 hours. The reaction conditions described in Abubakar *et al* stated that in each case the hydrolysis was continued for 24 hours. The paper does not describe any hydrolysis conducted for any period of time shorter than this. The degree of hydrolysis of the resulting hydrolysate is6.3%. The hydrolysate was inactivated using conditions as close as possible to those described by Abubakar *et al.* namely 96°C/10 min. I have read and refer to the declaration of Sophia Stathopoulos being filed with this declaration, which describes that hydrolysis.

11. The conclusion reached by Ms Stathopoulos and illustrated in the photographs is that the hydrolysate is of no commercial value. Although small and medium sized peptides in the hydrolysate which express ACE inhibiting activity are soluble because they possess no secondary or territory structure, the undigested protein and the very large protein fractions which have been denatured by the deactivating conditions are also present. The hydrolysate as a whole is not able to be further processed to sell as a commercial product.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willfull false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that willfull, false statements may jeopardize the validity/enforceability of the application or any patent issued thereon.

Dated: 18-03-2004 Signature: Julian Reid

Julian Robert Reid

This is the exhibit marked "JRR1" referred to in paragraph 2 of the annexed declaration of JULIAN ROBERT REID declared at Palmerston North this 18th day of March 2004 in relation to USSN 09/720,041.

CURRICULUM VITAE OF DR JULIAN R REID

Full name: Dr Julian Robert Reid

Present position: Senior Research Scientist

Present employer: Fonterra Research Centre Limited

Present work address: Private Bag 11029
Palmerston North
New Zealand

Academic qualifications: *(including year awarded)*
1990 BSc Hons (First Class), Massey University, Palmerston North, New Zealand
1994 PhD, Massey University, Palmerston North, New Zealand

Years as a practising researcher: 9

Honours/distinctions/membership of societies, institutions, committees:
(include both academic and end-user involvement)
1990 Massey University Scholar
1991-1994 New Zealand Vice Chancellor's Committee Scholar
1994-present Member, New Zealand Society for Biochemistry and Molecular Biology

Professional positions held: *(years, position, institution, activity)*
1978–1990 Research Technician, Chem/Biochem Dept., Massey University, Palmerston North
1994–1997 Research Scientist, NZ Dairy Research Institute, Palmerston North
1997–1998 Visiting Research Worker, NIZO Food Research, Ede, The Netherlands
2000–present Senior Research Scientist, Fonterra Research Centre, Palmerston North

Present research/professional speciality:
Enzymology of lactic acid bacteria; food-grade preparative isolation of proteins and peptides for sensory analysis; isolation and characterisation of bioactive peptides from protein hydrolysates; quantitative and qualitative analysis of peptides using LC/MS/MS. Identification of proteins in bovine milk and dairy microorganisms using proteomics. Protease inactivation and mode of action in enzyme modified cheeses.

Number of refereed publications:	15	
Number of patents:	3	
Number of significant publications not included in the above:	2	

Number of research qualifications at Level 7 NQF/Hons supervised to completion:

Number of post-graduate theses supervised to completion:	PHD:	Masters: 2
Number of post-graduate theses examined:	PhD:	Masters:

List of major achievements:

1. Major publications (in the last five years).

JR.

Reid, J.R. & Coolbear, T. (1999) Specificity of *Lactococcus lactis* subsp. *cremoris* SK11 proteinase, lactocepin III, in low-water-activity, high-salt-concentration humectant systems and its stability compared with that of lactocepin I. *Appl. Environ. Microbiol.* **65**:2947-2953.

Christensson, C., Bratt, H., Collins, L.J., Coolbear, T. Holland, R., Lubbers, M.W., O'Toole, P.W. & **Reid, J.R**. (2002) Cloning and expression of an oligopeptidase, PepO, with novel specificity from *Lactobacillus rhamnosus* HN001 (DR20). *Appl. Environ. Micobiol.* **68**: 254-262.

Coolbear, T., Crow, V.L., Holland, R., Liu, S.Q. & **Reid, J.R**. (2002) *Lactococcus* spp.; flavour development, *In* Roginski, H., Fox, P.F. & Fuquay, J.W. (Ed.) *Encyclopedia of Dairy Sciences*. Academic Press, New York. Vol 3:1520-1525.

Pillidge, C.J., Crow, V.L., Coolbear, T. & **Reid, J.R**. (2003) Exchanging lactocepin plasmids in lactococcal starters to study bitterness development in Gouda cheese. *Int. Dairy J.* 13:345-354

Middleton N., **Reid, J.R**., Coolbear, T & Jelen, P. (2003) Proliferation and intracellular glutathione in Jurkat T cells with concentrated whey protein products. *Int. Dairy J.* 13:565-573.

Reid, J.R. & Coolbear, T. Lactocepin: the cell envelope-associated endopeptidase of *lactococci*, *In* Barrett, A..J., Rawlings, N.D. & Woessner, Jr. (Ed.) *Handbook of proteolytic enzymes*. Second Edition. Academic Press, London, England (*in press*).

JR.